Broiler Carcass Bacterial Counts After Immersion Chilling Using Either a Low or High Volume of Water

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ABSTRACT A study was conducted to investigate the bacteriological impact of using different volumes of water during immersion chilling of broiler carcasses. Market-aged broilers were processed, and carcasses were cut into left and right halves along the keel bone immediately after the final bird wash. One half of each carcass pair was individually chilled at 4°C in a separate bag containing either 2.1 L/kg (low) or 16.8 L/kg (high) of distilled water. Carcass halves were submersed in a secondary chill tank containing approximately 150 L of an ice-water mix (0.6°C). After chilling for 45 min, carcass halves were rinsed with 100 mL of sterile water for 1 min. Rinses and chill water were analyzed for total aerobic bacteria (APC), *Escherichia coli*, *Enterobacteriaceae*, and *Campylobacter*. After chilling with a low volume of water, counts were 3.7, 2.5, 2.6, and 2.1 $\log_{10}$ cfu/mL of rinse for APC, *E. coli*, *Enterobacteriaceae*, and *Campylobacter*, respectively. When a high volume of chill water was used, counts were 3.2, 1.7, 1.6, and 1.8 $\log_{10}$ cfu/mL of rinse for APC, *E. coli*, *Enterobacteriaceae*, and *Campylobacter*, respectively. There was no difference in bacterial counts per milliliter of chill water among treatments. These results show that using additional water during immersion chilling of inoculated broilers will remove more bacteria from the carcass surfaces, but numbers of bacteria per milliliter in the chiller water will remain constant. The bacteriological impact of using more water during commercial immersion chilling may not be enough to offset economic costs.

Key words: broiler, immersion chilling, carcass contamination, carcass microbiology

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INTRODUCTION

During commercial poultry processing, eviscerated carcasses are cooled by either water immersion or cold air-blast (Brant, 1974; Thomson et al., 1974; Veerkamp, 1989). Immersion chilling has traditionally been the most popular method for cooling poultry in the United States because mechanical agitation along with air injection in chiller water produces economical and efficient carcass heat transfer. In spite of its efficiency, immersion chilling has been criticized because it requires large volumes of water, and bacteria may be transferred from one carcass to another in the common bath (Mead et al., 2000; James et al., 2005). Air chilling of poultry has been suggested as an alternative to immersion chilling to eliminate carcass cross contamination; however, research has shown that cross contamination can still occur during both dry and evaporative air chilling (Mead et al., 2000).

In 2001, the USDA's Food Safety and Inspection Service issued a final rule that limits the amount of water retained by poultry products as a result of carcass washing and immersion chilling (USDA, 2001a). This ruling indicated that excessive retained water in poultry could result in "a product becoming misbranded or economically adulterated" (USDA, 2001a). In addition, product water retention is not allowed unless the processing establishment demonstrates that absorbed water "is an unavoidable consequence of processing" required to meet the pathogen performance standards (USDA, 1996, 2001a). Poultry processing establishments are required to document the amount of water retained in chilled carcasses and parts and disclose this information on the product label (USDA, 2001b). The Food Safety and Inspection Service indicates that the water retention regulation will result in "consistency" in processing procedures and "greater uniformity" in poultry inspection (USDA, 2001b).

A number of comprehensive review articles on poultry chilling have been published (Brant, 1963, 1974; Thomson et al., 1974; Lillard, 1982; James et al., 2005). Previous research has shown that the microbiology of immersion-chilled poultry carcasses depends upon the volume of water in the chiller, chiller overflow, ratio of carcasses to water, and the level of bacteria present on the carcass before chilling (Blood and Jarvis, 1974). These same researchers conducted a commercial survey of poultry-chilling parameters and found that carcass bacteriology was not compromised when the amount of water used to

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immersion chill poultry was reduced from a high (8 L/kg of carcass weight) to a moderate range (3.6 to 4.8 L/kg of carcass weight), provided there was 12 to 46 ppm of total residual chlorine in the water (Blood and Jarvis, 1974). Processing variables could not be controlled during the survey, and various combinations of water level (0.99 to 13.8 L/kg of carcass), free chlorine (0.1 to 35 ppm), and water pH (7.00 to 8.54) were not consistently represented (Blood and Jarvis, 1974). Other researchers suggested that 2.5 L/carcass were microbiologically sufficient during immersion chilling if the total residual chlorine was maintained at 50 ppm (Mead and Thomas, 1973). Mulder et al. (1976) reported that counts of total aerobic bacteria (**APC**) and *Enterobacteriaceae* varied by <0.5 log cfu/g of skin when 2.5 or 3.5 L/carcass was used during immersion chilling, and there was no difference in prevalence of *Salmonella* recovered from chilled poultry skin (6.7 and 7.1% positive for 2.5 and 3.5 L/carcass, respectively). These data suggest that the amount of water used during immersion chilling could be reduced without compromising heat exchange and carcass microbiology, and this might be favorable from both an economic and regulatory standpoint.

With the industry trend toward processing larger broilers and the new developments in poultry chilling, many of the previous immersion chilling recommendations no longer apply. The present study was conducted to evaluate poultry carcass bacteria recovery and chiller water bacteria levels after immersion chilling with low (2.1 L/kg) or high (16.8 L/kg) volumes of water in a controlled environment.

MATERIALS AND METHODS

Broilers

Broiler chicks were hatched from commercially obtained eggs and grown to market age (56 to 63 d) on pine shavings in floor pens (5 × 8 m) in a controlled environment-type house. Birds were fed a nonmedicated, corn-soybean meal-based starter diet (3,100 kcal ME/kg, 23% CP) for 3 wk, followed by a nonmedicated grower diet (3,200 kcal ME/kg, 21% CP) for the remainder of the study. When birds were 5 wk of age, they were individually weighed, identified by a leg band, selected for BW, and gavaged with a 1-mL suspension containing 10^6 cells/mL of *Campylobacter*. Before inoculation, feed was removed from broilers for 4 h to assist in intestinal colonization. On the day of processing, feed but not water was withdrawn for the first 4 h, followed by an additional 8 to 10 h without both feed and water (total feed withdrawal of 12 to 14 h). All broilers were processed in the pilot plant processing facility at the Russell Research Center.

Processing

During each of 4 replications, 8 broilers were processed in 2 sequential batches of 4 birds. Broilers were placed into shackles by their feet and stunned using a brine stunner (model SF-7001, Simmons Engineering Company, Dallas, GA) set at 12 V pulse direct current (500 Hz) for 12 s. Stunned broilers were slaughtered using an automatic rotary blade (model SK-5, Simmons Engineering Company) to sever both carotid arteries and jugular veins. Carcasses were bled for 90 s, scalded at 55.6°C for 90 s in a single tank (model SGS-3CA, Stork-Gamco Inc., Gainesville, GA) and defeathered for 30 s using a single-pass mechanical picker (model D-8, Stork-Gamco Inc.). The head and feet were manually removed from each carcass. Carcasses were then transferred to the evisceration line where they were mechanically eviscerated (model PNT-24, Stork-Gamco Inc.) and washed in an inside-outside bird washer (4 s wash at 80 psi; model MBW-16, Stork-Gamco Inc.) with tap water (chlorine level 0.5 to 1.0 ppm). Immediately after the final bird washer, 1 carcass from each batch (2 per replication) was subjected to a whole carcass rinse (**WCR**). The WCR was performed by placing the carcass into a plastic bag with 100 mL of sterile water and shaking for 1 min. After shaking, carcasses were allowed to drain back into the bag and then discarded. The WCR were referred to as the prechill controls and were analyzed using the microbiological procedures described below.

The remaining carcasses (6 per replication) were cut into left and right halves along the keel bone and each half was tagged on the wing and weighed. One-half of each pair was individually chilled in a plastic bag containing either 2.1 L/kg (low) or 16.8 L/kg (high) of distilled water (4°C). No chlorine was added to the distilled water. Bags containing carcass halves and water were sealed with multiple cable ties and submersed in a secondary chill tank containing approximately 150 L of an ice-water mix (0.6°C). The secondary chill tank was agitated with compressed air. After chilling for 45 min, samples were removed from bags using clean sterile gloves and allowed to drip for 5 min. Approximately 100 mL of chill water from each bag was collected for microbiological analyses (replications 2 through 4 only). Each half was placed into a new bag with 100 mL of sterile water and subjected to a half-carcass rinse (**HCR**) for 1 min. After rinsing, halves were removed from the bags, deep breast muscle temperature was measured, and carcasses were then discarded.

Microbiological Analyses

The carcass rinses and chill water from each sample were analyzed for APC, *Escherichia coli*, *Enterobacteriaceae*, and *Campylobacter*. Because *Campylobacter* numbers on samples were low in the first replication, the decision was made to include bacteriological testing of the chill water in subsequent replications. Serial dilutions of the rinse diluent were made in 1% peptone. The APC were enumerated on plate count agar (Becton Dickinson, Sparks, MD). One-tenth of 1 mL from a serial dilution of rinse diluent was plated in duplicate on the surface of agar, spread, and incubated at 35°C for 48 h prior to counting the resulting colonies. *Escherichia coli* counts were made by

Table 1. Bacterial counts (means ± SE) recovered from carcass rinses before immersion chilling (prechill control)

Bacteria	$\log_{10}$ cfu/mL	$\log_{10}$ cfu/carcasses
Total aerobic	5.2 ± 1.1	8.5 ± 1.1
Escherichia coli	4.5 ± 0.6	6.8 ± 0.6
Enterobacteriaceae	3.8 ± 0.8	6.1 ± 0.8
Campylobacter	4.8 ± 0.1	7.1 ± 0.1

plating 1 mL from a serial dilution of rinse diluent onto duplicate *E. coli* Petrifilm plates (3M Health Care, St. Paul, MN). Petrifilm plates were incubated at 35°C for 24 to 48 h, and blue colonies closely associated with entrapped gas were counted as *E. coli*. *Enterobacteriaceae* were enumerated using duplicate pour plates of violet red bile glucose agar containing 1 mL from a serial dilution. All violet red bile glucose agar plates were overlaid with approximately 5 mL and then incubated at 35°C for 48 h. Plates with the typical presumptive *Enterobacteriaceae* colonies were counted and reported. *Campylobacter* was enumerated by plating 0.1 mL from the serial dilutions onto Campy Blood agar (Blaser) and incubating the plates at 42°C for 36 h in a microaerophilic environment (5% O_2, 10% CO_2, and balance N_2). The colonies characteristics of *Campylobacter* were counted. Each colony type identified as *Campylobacter* was confirmed for genus by examination of cellular morphology and motility on a wet mount under phase-contrast microscopy. Each colony type was further identified as *Campylobacter* spp. using INDX-Campy (jcl) culture confirmation test (Integrated Diagnostics, Baltimore, MD).

Statistical Analysis

All statistical analyses were performed on data after log transformation. Data were analyzed by the GLM procedure of the SAS/STAT program (SAS Institute, 1999) using treatment (low or high water volume) and replication as main effects. All first order interactions were tested for statistical significance ($P < 0.05$) using the residual error mean squares. Bacterial counts were also analyzed using the paired t-test in the means procedure of SAS (SAS Institute, 1999).

RESULTS AND DISCUSSION

The mean logarithmic microbial counts for prechill control carcasses expressed as both $\log_{10}$ cfu/mL of rinse and $\log_{10}$ cfu/carcass are shown in Table 1. Before chilling, counts recovered from carcasses were 5.2, 4.5, 3.8, and 4.8 $\log_{10}$ cfu/mL of rinse or 8.5, 6.8, 6.1, and 7.1 $\log_{10}$ cfu/carcass for APC, *E. coli*, *Enterobacteriaceae*, and *Campylobacter*, respectively. Bacterial counts for APC, *E. coli*, *Enterobacteriaceae*, and *Campylobacter* recovered from broiler carcass rinses after immersion chilling in a low (2.1 L/kg) or high (16.8 L/kg) volume of water are shown in Table 2. Overall, immersion chilling reduced levels of APC, *E. coli*, *Enterobacteriaceae*, and *Campylobacter* by 1.2 to 3.0 log units. *Escherichia coli* and *Campylobacter* levels in HCR declined the most after immersion chilling, with a 2.8 and 3.0 log difference, respectively (Table 2). These data agree with previous research, which reported that counts of APC, *E. coli*, and *Campylobacter* recovered from broiler carcasses decreased by more than l.0 log after immersion chilling (Mead and Thomas, 1973; Izat et al., 1988; Blank and Powell, 1995; Cason et al., 1997; Bilgili et al., 2002; Northcutt et al., 2003). Commercial immersion chilling has also been found to reduce the numbers of *Enterobacteriaceae* recovered from chicken carcasses by 0.8 log/cm^2 (Cox et al., 1975). Previous research reported that 87.2 to 96.7% of the *Enterobacteriaceae* isolates recovered from chilled carcasses were from the genus *Escherichia*, and these findings explain the similarities in *E. coli* and *Enterobacteriaceae* data reported in this present study (Cox et al., 1975).

The initial hypothesis for the study was that immersion chilling in a controlled environment with a high volume of water would result in increased exposure to fresh water, and more bacteria would be removed from the carcasses. Thus, a high volume of chill water could result in lower counts recovered in the subsequent HCR, but higher counts per milliliter in chill water. The data seem to support this concept. Using a high volume of water during chilling decreased APC, *E. coli*, *Enterobacteriaceae*, and *Campylobacter* counts recovered from carcasses by 0.5, 0.8, 1.0, and 0.3 log units, respectively, when compared with counts recovered from carcasses chilling in a low volume of water (Table 2). It is important to note that

Table 2. Bacterial counts recovered from carcass rinses after immersion chilling with either a low (2.1 L/kg) or high (16.8 L/kg) volume of water[1]

	Rinse			Carcass		
Bacteria	Postchill high volume	Postchill low volume	*P*-value	Postchill high volume	Postchill low volume	*P*-value
	($\log_{10}$ cfu/mL)			($\log_{10}$ cfu/carcass)		
APC[2]	3.2 ± 0.4	3.7 ± 0.3	0.0006	5.5 ± 0.4	6.0 ± 0.3	0.0006
Escherichia coli	1.7 ± 0.2	2.5 ± 0.2	0.0001	4.0 ± 0.2	4.8 ± 0.2	0.0001
Enterobacteriaceae	1.6 ± 0.2	2.6 ± 0.1	0.0001	3.9 ± 0.2	4.8 ± 0.1	0.0001
Campylobacter	1.8 ± 0.2	2.1 ± 0.2	0.0004	4.1 ± 0.2	4.4 ± 0.2	0.0004

[1]Counts are expressed as $\log_{10}$ cfu ± SE recovered from each milliliter of rinse ($\log_{10}$ cfu/mL) or recovered from the entire carcass ($\log_{10}$ cfu/carcass).

[2]APC = total aerobic bacteria.

Table 3. Bacterial counts recovered from chiller water when carcass halves were cooled using either a low (2.1 L/kg) or high (16.8 L/kg) volume of water[1]

Bacteria	Chill water			Carcass equivalent		
	High volume	Low volume	*P*-value	Chill water high volume	Chill water low volume	*P*-value
	($\log_{10}$ cfu/mL)			($\log_{10}$ cfu/carcass equivalent)		
APC[2]	4.3 ± 0.2	4.3 ± 0.2	NS[3]	8.4 ± 0.2	7.6 ± 0.2	0.0001
Escherichia coli	3.5 ± 0.2	3.3 ± 0.2	NS	7.7 ± 0.2	6.6 ± 0.2	0.0001
Enterobacteriaceae	3.4 ± 0.2	3.2 ± 0.2	NS	7.5 ± 0.3	6.5 ± 0.2	0.0001
Campylobacter	2.7 ± 0.2	3.0 ± 0.4	NS	6.7 ± 0.2	5.7 ± 0.2	0.0001

[1]Counts are expressed as $\log_{10}$ cfu ± SE recovered from each milliliter of chill water ($\log_{10}$ cfu/mL) or recovered from the entire carcass ($\log_{10}$ cfu/carcass equivalent).

[2]APC = total aerobic bacteria.

[3]NS = $P > 0.05$.

although statistically significant, the 0.5 log APC and 0.3 log *Campylobacter* reductions in HCR achieved after high volume of immersion chilling would not have a practical impact.

Table 3 shows counts of bacteria ($\log_{10}$ cfu/mL of chill water and $\log_{10}$ cfu/carcass equivalent) recovered from chiller water after carcasses were cooled with either a low or high volume of water. When the data were expressed on a per milliliter basis, there was no difference ($P > 0.05$) in the levels of any of the bacteria recovered in chiller water regardless of volume (Table 3). Comparable numbers of bacteria per milliliter of chiller water appear to support the conclusions of Lillard (1988, 1989), who evaluated the bacteriological impact of rinsing the same carcasses multiple times with sterile peptone solution (1%). While she observed a pattern of slight decline in bacteria counts after successive 60-s carcass rinses, Lillard (1988, 1989) recovered large numbers of bacteria even after 40 consecutive rinses of the same carcass. Because she found comparable numbers of bacteria per milliliter of carcass rinse, Lillard (1989) concluded that "all bacteria are not removed by a single or even by 10 consecutive rinses."

When the counts of bacteria recovered from the chiller water were expressed as carcass equivalent, the high volume of chiller water contained significantly ($P < 0.05$) more bacteria than the low volume of chiller water. Counts recovered from the high volume of chiller water were 0.8, 1.1, 1.0, and 1.0 $\log_{10}$ cfu/carcass higher than the counts recovered from the low volume of chiller water for APC, *E. coli*, *Enterobacteriaceae*, and *Campylobacter*, respectively (Table 3). This demonstrates that increasing the amount of water during chilling resulted in the removal of additional bacteria from carcasses when the bacteria originated in inoculated broilers.

Analyses of the data from the present study demonstrate that there may be a relationship between volume of water used during immersion chilling and the numbers of bacteria recovered from chilled poultry carcasses. Previous reports indicate that using more water during immersion chilling will improve efficiency of carcass heat transfer (Veerkamp, 1989), but the present study suggest that the small bacteriological reduction may not be economically beneficial to the commercial poultry industry. Data from the present study support the conclusion that increasing the volume of water without controlling other aspects of immersion chilling (water temperature, carcass dwell time, initial carcass bacteria load, chlorine level) will provide only a minor improvement in the bacteriological condition of poultry.

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